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STUDIES ON THE FIXATION OF GORAN TANNINS ON CHEMICALLY MODIFIED COLLAGEN*

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SUMMARY

Studies on the fixation of original and the purified goran liquors on modified collagen showed no direct correlation between the fixation of tan and the hydrothermal stability. The results obtained have been discussed in the light of current thoughts on the mechanism of vegetable tannage. From the results =CONH-groups are considered to play a major role in the fixation of goran tannin through hydrogen bonding.

In vegetable tannage, inspite of extensive investigations, the nature of reactions taking place is still undecided. Until recently, the mechanism of reaction was considered to be "mutual co-precipitation" between negatively charged tannin particles and positively charged collagen,[1] which has been discarded nowadays, since principal tannins are not charged under conditions of tanning.

The possible sites for binding of tannin are (a) peptide groups (keto-imide groups), (b) ionic groups, (c) hydroxyl groups, (d) amide groups. These groups are theoretically capable of bond formation with phenolic OH's or etheric O's of the tannin polyphenols. Roux[2] and Sykes and Roux[3] reported that the qualitative changes in the affinity relationship between collagen and a condensed tannin (wattle) brought about by chemical modification of the latter are greater than those caused by inactivating specific groups in collagen. According to them, the chemical modifications of collagen only brought about changes which could be attributed to differences in the swelling characteristics. Sykes[4] has also reported that the hydrothermal stability of the tanned collagen is dependent on the amount of tannin fixed, rather than on fixation of mimosa tannins at any particular site in the collagen. The present work was undertaken to get a clear picture of the function of the various groups in collagen in the reaction with goran tannins (*Ceriops roxburghiana*) which is abundantly available in India and which has been found to be the best tanning material of the three mangroves examined.[5]

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Table 1

TANNING WITH GORAN AQUEOUS INFUSION

Substrate		Salt added (% w/v)	pH of tanning	Swelling	Tan fixed %	Yield %	T_s (°C)	Penetration 3 days	Penetration 11 days	Penetration 18 days
Deaminated collagen	D_1	—	2·5	130·3	41·9	168·6	87	Full	Full	Full
	D_2	—	4·5	159·6	42·9	186·2	86	¾	,,	,,
	D_3	—	6·5	115·3	44·1	174·3	81	Almost full	,,	,,
	D_4	5	2·5	131·2	43·4	172·2	86	Full	,,	,,
Methylated collagen	M_1	—	2·5	233·0	64·3	198·3	74	⅙	⅓	⅔
	M_2	—	4·5	255·4	57·6	214·2	84	⅙	⅔	Almost full
	M_3	—	6·5	198·8	52·7	192·6	89	⅙	⅔	,,
	M_4	5	2·5	216·7	53·9	243·7	82	½	Full	Full
Acetylated collagen	A_1	—	2·5	144·5	47·2	170·1	75	¼	½	⅔
	A_2	—	4·5	146·9	49·8	173·4	95·5	⅓	Full	Full
	A_3	—	6·5	148·0	46·4	173·8	88	⅓	Almost full	,,
	A_4	5	2·5	139·0	46·3	174·2	82	⅔	Full	,,
Intact collagen (Blank)	B_1	—	2·5	142·1	47·1	213·6	66·5	¼	½	⅔
	B_2	—	4·5	111·3	43·8	168·9	95	⅓	Full	Full
	B_3	—	6·5	135·9	47·6	180·3	95	⅓	Almost full	,,
	B_4	5	2·5	112·1	43·3	169·0	93·5	½	Full	,,

Experimental

Collagen used is this work was prepared by the method of Sykes.[4] Both grain and flesh layers of limed buff hide were removed by splitting. The piece was then delimed and washed free of salts, dehydrated with several changes of acetone and cut into small pieces (*ca* 1×5 cm.). The pelts were then mixed and divided into 4 lots. All the lots except one were chemically modified. Deamination, acetylation and esterification were carried out following the methods of Bowes and Kenten,[6] Green *et al*[7] and Burton *et al*[8] respectively. All the modified pieces were then dehydrated with acetone.

The dehydrated pieces (weighed) were then rehydrated in 100 times their weight of distilled water adjusted to pH 2·5, 4·5 and 6·5 with HCl or NaOH. To the lot adjusted to pH 2·5, 5% (w/v) sodium chloride was added to depress swelling. After 24 hours the pieces were removed, blotted to remove surplus water, weighed and the degree of swelling was calculated as per Sykes.[4]

Goran liquor (2·5% tan strength) was prepared and adjusted to the same pH values (2·5, 4·5 and 6·5) as those of the soak liquor using NaOH and HCl. In the case where the tan liquor was adjusted to pH 2·5, 5% NaCl (w/v) was added. The liquor/dry pelt ratio was kept at 30:1 and tanning was carried out for 18 days changing the liquor on the 7th and 13th days. The pH of the liquor was kept constant throughout. Penetration was noted periodically. After tanning, the pieces were removed and blotted to remove the adhering surplus liquor and weighed. A small piece was then taken for T_s determination in each case and the remaining tanned pieces were dried and then analysed. The results are shown in Table 1.

These sets of experiments were repeated using aqueous infusion of purified goran (prepared by the method of Roux)[9] under identical conditions and the results obtained are given in Table 2.

Discussion

The first direct demonstration of the participation of the basic groups in tannin fixation was adduced by Thomas and his group.[10] who showed that deamination of collagen decreases its capacity for irreversible fixation of tannins in tanning with solutions of pH values lower than the isoelectric point of deaminated collagen. Lollar and his group[11] concluded that amino groups do not play any prominent role in the vegetable tannage. But this has been criticised by Gustavson[12] who stated that in view of the changes in the nonionic groups of collagen occurring in the deamination process, the statement of Lollar does not represent the actual facts. Bowes and Kenten[13] on the other hand, took the opposite attitude and ascribed the total fixation of tannins to the basic protein groups. They showed that for the mimosa tannins, there was only a slight decrease in the uptake of tannins by the deaminated collagen in the pH range 4-7.

It has been stated[14] that by methylation (esterification) of collagen in methanol at very low pH the cationic protein groups are set free completely and that the high swelling brought about by methylation might be due to the extensive rupture of the interchain hydrogen bonding.

The present work has studied the inactivation of amino, carboxyl and hydroxyl residues in the collagen. It was observed that nontans have got very little effect on shrinkage temperature at the natural pH (4·5) of the modified collagen as both purified and original goran liquors showed almost the same readings whereas these (nontans) have got some appreciable effect at the natural pH so far as the fixed tan is concerned.

The fixation of both purified and original tannin by the deaminated pelt was found to be slightly less than that by intact pelt at all pH levels. These findings are in agreement with those of Lollar and his group[11] and Page.[15] As suggested by Shuttleworth,[16] this could be due to the removal of groups which hydrogen-bond to the tannin and to reduced accessibility of active protein groups to large tannin molecules which require multipoint attachment in order to be classified as fixed tannins. Lollar and coworkers[11] stated that the degree of tannage of normal and deaminated collagens confirms the previous observations that the fixation of quebracho tannin by the hide at its isoelectric point is not greatly affected by deamination. Our work on goran tannins also agrees well with these findings. This supports the view that the affinity of the natural tannins for the free amino groups of the proteins is not the dominent phenomenon in tannage. In case of goran (both purified and original) the deaminated pieces showed very slight increase in fixed tan, with increase in pH. The fixation of original liquor was slightly more than that of the purified liquor in the tanned deaminated pelt at all pH levels indicating that nontans play a certain role in the fixation of the deaminated pelt. But the data of Page[15] showed that tanned deaminated hide despite its low degree of tannage has slightly higher shrinkage temperature (2°) than normal leather. But in our work, deaminated pelts at pH 2·5 (both with and without the addition of salt) showed higher shrinkage temperature than the intact pelts in both the liquors. But at pH 4·5 (normal pH) both original and purified liquors showed much less T_s. At pH 2·5 addition of salt did not decrease the swelling and T_s in both the liquors. From the results it could be concluded that salt has got no positive effect on swelling of the deaminated pelt and shrinkage temperature of the tanned deaminated pelt. It was observed from the data that the T_s in intact collagen tanned at pH 2·5 is less than that of tanned deaminated pelt at the same pH. The rate of penetration, however, shows that even after 18 days tanning the penetration was not complete in case of intact pelt at pH 2·5, which might presumably be due to the excessive swelling, but this is not so in the case of deaminated pelt.

Swelling of the deaminated pelt was found to be independent of pH and it was found to be maximum at pH 4·5. No correlation could be drawn between swelling and fixed tan of the deaminated pelt.

Penetration of the tan liquor through deaminated pelt was found to be the quickest and even in 3 days the penetration by both purified and original liquors was complete at almost all the pH levels. The quick penetration might be due to the rupturing of crosslinking hydrogen bonds of collagen during deamination

Table 2

TANNING WITH AQUEOUS INFUSION OF PURIFIED GORAN

Substrate		Salt added (% w/v)	pH of tanning	Swelling	Tan fixed %	Yield %	T_s (°C)	Penetration		
								3 days	11 days	18 days
Deaminated collagen	D_1	—	2·5	130·4	38·4	160·4	83	Almost full	Full	Full
	D_2	—	4·5	151·8	39·4	164·8	87	"	"	"
	D_3	—	6·5	117·9	39·7	171·6	88	Full	"	"
	D_4	5	2·5	131·2	34·5	151·3	82	"	"	"
Methylated collagen	M_1	—	2·5	231·0	47·5	162·8	70	Streak	Streak	⅛
	M_2	—	4·5	247·9	42·5	168·5	80	"	"	⅛
	M_3	—	6·5	192·1	37·6	158·7	91	"	"	⅛
	M_4	5	2·5	203·6	41·9	164·9	79·5	⅛	½	Full
Acetylated collagen	A_1	—	2·5	143·6	46·4	174·6	72	Streak	½	Almost full
	A_2	—	4·5	155·4	43·7	169·4	93·5	½	Almost full	Full
	A_3	—	6·5	153·8	50·0	191·6	79	⅓	½	"
	A_4	5	2·5	141·4	41·6	173·8	81	⅔	Almost full	"
Intact collagen (Blank)	B_1	—	2·5	141·8	47·9	176·9	75	Streak	½	Almost full
	B_2	—	4·5	126·6	43·1	171·3	94	"	⅔	Full
	B_3	—	6·5	148·7	39·3	163·6	76	¼	½	"
	B_4	5	2·5	122·5	43·3	169·7	80	½	Almost full	"

and thereby facilitating tannins of different particle size to penetrate through the pelt. The feel of the leather pieces was very soft as compared to other pieces and the reason might be the same as stated before.

In the present experiment after the esterification the pelt pieces were found to be very hard and the penetration of the tan liquor through the pelt was also very slow. The penetration was found to be incomplete even in 18 days time in all the cases except the one where salt was added to suppress swelling. The purified tannins showed much less penetration than the original goran liquor. The lack of penetration might be due to excessive swelling of the pelt. The fixation of tan by the esterified collagen in case of original liquor is much more than the maximum fixation obtained by intact collagen at the point of its maximum swelling and this finding is in agreement with that of Gustavson.[14] As there was not much of penetration in most of the cases in the case of purified tannin and as the fixation was found to be appreciably less, it seems probable that nontans play an important role in penetration and fixation. Because of imperfect penetration, T_s of most of the pieces was found to be less than that of tanned intact collagen. Rupturing of hydrogen bond because of excessive swelling and incomplete penetration of liquor through the pelt has made the T_s low. In case of esterified collagen fixation was found to increase with increase in pH. The methylated pieces became harder after the completion of tannage. The swelling of the acetylated pelt was found to be more than that of the intact pelt at all pH values in both the liquors. But the fixed tan of the former showed almost the same reading as that of the latter in both the liquors. Tan fixed by the acetylated pelt was found to be independent of swelling and pH. As expected, at almost all the pH levels the results of both acetylated and intact pelt were found to give the same trend. High T_s of acetylated pelt for both the original and purified liquors at pH 4·5 is interesting. Penetration of acetylated and intact pelts was found to be almost the same.

From the above experiments, it could be concluded that amino groups do not play a great role in the fixation of goran tannin. The methylated pelts were so swollen and hard that penetration was incomplete even in 18 days tanning, in most cases. Nevertheless, the fixation was found to be highest, which might presumably be due to more of hydrogen bonding sites made available during methylation. So far as acetylation of pelt is concerned appreciable change was not observed in the fixation and shrinkage temperature. It seems probable that hydroxyl groups also do not play a significant part in the fixation of condensed tannins. From the experiments carried out with hydrated polyamide and hide powder under identical conditions, it was observed that hide powder fixed 59% and Ultramid 39% of goran tannins.[17]

As the only reactive groups of any significance present in the polyamide is the keto-imide (-CONH-) group, high fixation of goran tannin by the polyamide supports the work of Gustavson that peptide bond can bind tannins if it is present in the uncompensated state. As other groups like amino and hydroxyl in

the collagen do not fix goran tannin to a considerable extent, it seems probable that -CONH- group play a major role in the fixation of goran tannin through hydrogen bonding.

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